Development of Methods for Extracting DNA from Bacterial Spores

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RESEARCH OBJECTIVES

The Federal Bureau of Investigation (FBI) has been mandated to develop and establish a wide range of unprecedented capabilities for providing scientific and technical forensic services to investigations involving hazardous chemical, biological and radiological materials, including extremely dangerous chemical and biological warfare agents. Presently, there are many technological shortfalls that must be addressed in order to achieve the required capabilities.

Certain microbial spores, such as those of *Bacillus anthracis*, may be attractive to terrorists as weapons of mass destruction because they are easily produced, easily transported, resistant to environmental and other forms of degradation, and extremely hazardous. To date, spore structural signature assays have not been developed for use in a forensic scenario.

The objective of this project was to develop methods for extracting PCR-amplifiable DNA from endospores of *Bacillus anthracis* in sufficient quality and quantity for use in DNA-based forensic assays.

APPROACH

At the sponsor's special request, we had to avoid any toxic and hazardous chemicals or sophisticated instrumentation. Therefore, we needed to develop:

- rapid spore disruption methods;
- DNA capture and purification methods for spore extracts;
- DNA quantitation methods for spore extracts;
- assays and protocols to determine the efficacy and efficiency of DNA extraction and the reproducibility of results;
- methods for the analysis of forensic sample matrices.

ACCOMPLISHMENTS

We prepared a critical state-of-the-art review of the chemical structure of bacterial endospores and the current methods and their limitations with regard to spore disruption, DNA extraction and purification. We identified sample preparation problems and made recommendations for the development of improved, reliable and reproducible methods that can be employed under real world forensic conditions. We developed methods used to prepare purified spores, optimized the ballistic disruption technique for crude spore preparation, and modified protocols for DNA capturing and purification. Also, we adapted qualitative methods for spore disruption verification and used fluorescence detection for rapid quantitative DNA concentration measurement. Using *B. anthraci*-spiked soil samples, we verified the efficacy and efficiency of the developed protocols. We also used a wide range of physical and chemical spore damaging factors and tested the developed methods. In a two-day session, we trained molecular biologist colleagues from the FBI in applying our standard operating procedures (SOPs).

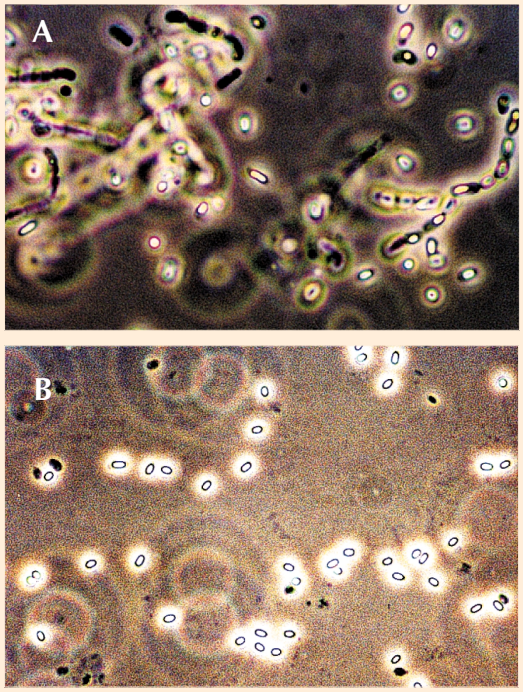

Figure 1. *B. anthracis* strain AMES showing vegetative cells, cells with spores (A) and free fully mature bacterial endospores (B) by phase contrast microscopy.

SIGNIFICANCE OF FINDINGS

The developed protocols enable the FBI and other agencies to handle real world forensic samples and extract PCR-amplifiable DNA from bacterial endospores with high reproducibility under field conditions. The SOPs are included in the final report, which fully documents the project.

ACKNOWLEDGEMENTS

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